POLYPLOIDY IN ENKIANTHUS (ERICACEAE)

HALLY J. SAX

ENKIANTHUS is a comparatively small genus belonging to the Ericaceae. According to Rehder (1940, 1949) there are about ten species, although more than double that number have been reported. Many of the so-called species which were described in isolated instances were considered by Rehder (1940) and Wilson (1907, 1929) to be geographical forms of species already described.

Most species of *Enkianthus* are attractive shrubs or small trees growing wild in southeastern Asia, Japan, southern, central and western China, and the Himalayas. Fang (1935), in a preliminary study of the Chinese species, said that there is not a single species in the northern provinces of China. He commented that only six of the twenty-one species described are valid.

The confusion in the classification of the genus is not surprising. J. D. Hooker (1879) wrote, "The Eastern genus *Enkianthus* presents four types of structure which almost indicate as many genera and would do so if the species had not been united by habit and if the characters were associated instead of applying to one species only."

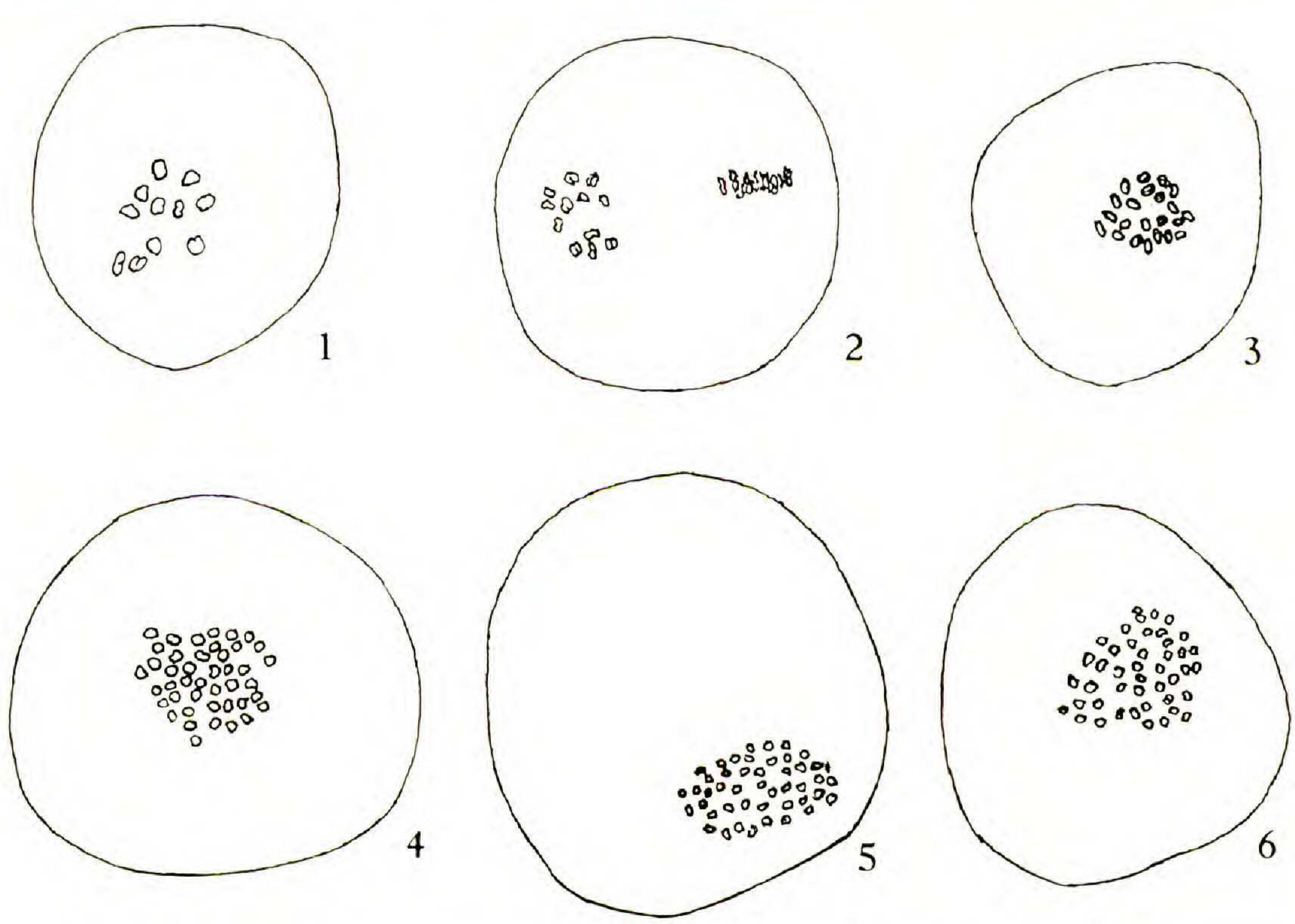
The number of chromosomes in *Enkianthus* was studied to determine if there was polyploidy in the genus. The variety in the structure of the flowers in the small number of species of *Enkianthus*, as well as their limited distribution, would offer possibilities of interesting combinations. Stomatal size and shape were also determined to learn if there was any correlation between stomatal size and chromosome number in the genus.

Three species of *Enkianthus* with some varieties are growing in the Arnold Arboretum: *E. campanulatus* (Miq.) Nichols, with vars. *albiflorus* Mak. and *palibinii* Bean; *E. perulatus* (Miq.) Schneider; and *E. subsessilis* (Miq.) Mak. Some of these on Bussey Hill were planted as early as 1890. A more recent and successful planting of the same species was made on low ground along the meadow road to the administration building. *Enkianthus cernuus* (Sieb. & Zucc.) Mak. var. *rubens* (Maxim.) Mak. is now being propagated in the Arboretum greenhouse. The chromosome numbers of the species of *Enkianthus* in the Arnold Arboretum were studied from meiotic divisions in the pollen mother cells, from the divisions of somatic cells in the ovary and from regenerating parenchyma of the stem. Very good preparations of divisions in both metaphase and anaphase in the dividing egg cell were also obtained.

The regenerating parenchyma is obtained according to the bark-peel method developed by Sax (1959). A longitudinal section of the bark is removed from the growing stem. The wound is covered with a strip of polyethelene film and left for several days. The newly developed tissue is then sliced off and fixed. The anthers, ovules and bark tissue were fixed in alcohol-acetic solution for twenty-four hours. The material was then changed to 95 per cent alcohol. Pieces of these tissues were macerated and smeared on a slide and stained with acetocarmine. Camera lucida drawings were made from some of the slides. I wish to thank Dr. Karl Sax for making the bark peels and for checking most of the counts.

The chromosomes were counted in the divisions of the pollen mother cells in *Enkianthus subsessilis* and several plants of *E. campanulatus* including the varieties *albiflorus* and *palibinii*. The chromosomes were also counted in the somatic tissue of the ovules. In the case of *E. perulatus*, counts were made in the somatic tissue in the ovule where it was possible to obtain several very good division figures where the chromosomes could be counted. Counts were also made from the regenerating bark in *E. perulatus* and *E. campanulatus*, where very good preparations of the divisions in the large parenchyma cells were obtained. In all cases several very good preparations were obtained.

Enkianthus subsessilis was found to be a diploid with 11 chromosomes in the meiotic divisions in the pollen mother cells. They were very clear in the preparations and could easily be counted in both the metaphase and anaphase stages. The camera lucida drawings show clearly 11 bivalent chromosomes in the first metaphase of the dividing nucleus of the pollen mother cell (FIG. 1). The 11 anaphase chromosomes are very clear in



FIGS. 1–6. Chromosomes of *Enkianthus*. 1, *E. subsessilis*, first meiotic metaphase; 2, *E. subsessilis*, second meiotic metaphase; 3, *E. perulatus*, somatic metaphase; 4, *E. campanulatus*, first meiotic metaphase; 5, *E. campanulatus*, second meiotic metaphase; 6, *E. campanulatus* var. *albiflorus*, second meiotic metaphase. All figures approximately × 1000.

FIG. 2 just as they are oriented at the poles. There were 22 chromosomes in the somatic tissue of the developing ovule (TABLE I).

Enkianthus perulatus is also a diploid. The count in this case was made in the somatic tissue of the ovule and in the parenchyma of the regenerating bark. Very clear counts in both metaphase and anaphase were obtained. There were 22 chromosomes in the somatic tissue, as is shown in FIG. 3 which was drawn from parenchyma tissue obtained by the bark-peel technique.

Enkianthus campanulatus is a high polyploid — presumably an octoploid — with approximately 88 chromosomes. There were about 44 chromosomes in the divisions of the pollen mother cells of several plants labeled as this species. Multivalent chromosomes were present and an occasional univalent was seen. The divisions were regular, suggesting some degree of allopolyploidy. Only in a few cases were there any lagging chromosomes. Camera lucida drawings were made of the dividing nuclei from several of these plants; all show approximately 44 chromosomes (FIGS. 4, 5).

Enkianthus campanulatus var. *albiflorus* is also an octoploid with $n = 44$ at meiosis. Multivalent associations of three and four chromosomes were seen in some cases, but bivalents predominated. The divisions were regular except in rare instances (FIG. 6). The number of chromosomes in the regenerating bark and in the ovule was over 80, the counts varying from 82 to 89. The chromosomes were small and it was difficult to be exact with such large numbers. TABLE I shows the number in each case.

The two diploid species belong in different sections of the genus, while the octoploid is in a third section. Polyploidy is not necessarily associated

TABLE I. **Chromosome Numbers in Enkianthus**

SPECIES	ARBORETUM NO.	MEIOTIC	SOMATIC	ZONE *
E. campanulatus	14528–1–D	44±	80–87	IV
	14528–1–G	44±		
	14528–1–C		88±	
	23001–1–C		88±	
	7692	44		
	507–58–D		88±	
	507–58–E		78–87	
var. *albiflorus*	14783–E	44±		IV
	7039–3–A	44±		
	14783–G		88±	
E. perulatus	20153–B		22	V
	10128–B		22	
	3591		22	
E. subsessilis	2449–B	11	22	V
	2449	11		

* A map of the climatic zones is found in Rehder (21). The zones are numbered from the North Pole southward. They are irregular according to the prevailing climate.

with taxonomic diversity. The fact that the octoploid species shows predominantly bivalent chromosomes at meiosis indicates that it is an amphiploid with different genomes.

Chromosome numbers have been reported for many genera of the Ericaceae (Darlington and Janaki-Ammal, 1945). Longley published the chromosome numbers of *Vaccinium*, a polyploid series with the basic number 12. Hagerup (1928) studied several genera: *Calluna vulgaris*, with 16; *Kalmia*, 24 and 48; *Cassiope hypnoides*, 48; *Erica*, 24; *Chimaphila umbellata*, 26; *Ledum*, 26 and 52; *Ramischia*, 38; and *Pyrola*, 46 and 92.

Wanscher (1933) reported 24 chromosomes in *Phyllodoce*; Wulff (1939), 22 chromosomes in *Moneses uniflora*; Maude (1940), 24 in *Daboecia cantabrica*; Newcomer (1941), 24 in *Gaultheria procumbens*; Callan (1941), 36 chromosomes in *Bruckenthalia*, 22, 44, 66 in *Pernettya*, 22, 44, 88 and 26 in *Gaultheria*, 24 in *Pieris*, 48 in *Andromeda*, 24 in *Leucothoë*, 24 in *Erica*, and 26 in *Arbutus*, *Arctostaphylos*, and *Ledum*. Hagerup (1941) counted 60 chromosomes in *Enkianthus campanulatus*, 26 in *Chimaphila*, and 52 in *Ledum*. Baldwin (1942) found 24 chromosomes in *Oxydendrum arboreum*.

Sax (1930) reported the number of chromosomes in *Rhododendron* as 26 for many species and 52 for two higher polyploids, the latter being native to North America. Nakamura (1931) found 26 to be the chromosome number for nine more species of *Rhododendron*. Janaki-Ammal (1950) reported *Rhododendrons* with $4x$, $6x$, $8x$, and $12x$ chromosomes in the high Himalayas in Eastern Asia, polyploidy increasing with altitude. Li (1957) also found 26 chromosomes in all the species of the "luteum" section of the azaleas, excepting the tetraploid *R. calendulaceum* and its hybrids and one triploid.

Thus, with few exceptions (*Calluna vulgaris*), the basic numbers of chromosomes are 11, 12, and 13. Polyploidy is prevalent in many genera, e.g., *Vaccinium*, *Pernettya*, *Gaultheria*, *Pieris*, and *Ledum*. As noted above, Hagerup (1941) reported 60 chromosomes for *Enkianthus campanulatus*, considering it a high polyploid, and suggesting 12 as the basic number both for *Enkianthus* and for the family.

Enkianthus has a very narrow distribution: southeastern Asia, central and western China, and Japan. The polyploid *E. campanulatus* is more widely distributed than the diploids, which have a narrow range and are limited to the warmer parts of China and Japan. Yet, compared with the *Rhododendrons*, which are native to a large part of both hemispheres, the polyploid *E. campanulatus* which extends from northern Japan to southern and central China and to the Himalayas in West China is a naturally limited species. However, when in cultivation it grows very well in the North Temperate Zone in Europe and the United States. The diploid species *E. perulatus* and *E. subsessilis*, as well as *E. cernuus* var. *rubens*, are also able to grow fairly well when introduced as far north as Boston, Massachusetts.

Stomatal size was also studied in three species of *Enkianthus*. The epidermis was peeled from the leaf and mounted on a slide in a drop of acetocarmine. Camera lucida drawings were made from the mounted epidermis. The diploids *E. perulatus* and *E. subsessilis* showed very little difference in size, but there was some difference in shape. Although no appreciable difference in size was found between the stomata in *E. perulatus* and *E. subsessilis*, there is a striking difference in size between these diploids and the high octoploid *E. campanulatus*. The stomata of *E. campanulatus* are very much larger than those of the diploids, as was noted by comparing the figures in camera lucida drawings of the stomata of these species. There is a very definite correlation between stomatal size and number of chromosomes in the species of *Enkianthus* studied.

Counts were made of the fertile and sterile pollen grains in the different species. Usually the fertility was high, around 85 per cent, but as low as 35 per cent was found. These latter cases were not consistent for any of the species studied and may not be typical, the aberrance having been caused, perhaps, by factors not studied.

SUMMARY

There are 11 meiotic chromosomes in *Enkianthus subsessilis*, 22 somatic chromosomes in *E. perulatus* and *E. subsessilis*. Both are diploid species.

The meiotic chromosomes in *Enkianthus campanulatus* and var. *albiflorus* are 44, and there are about 88 ± chromosomes in the dividing somatic cells. *Enkianthus campanulatus* and its varieties are octoploids.

The stomatal size in *Enkianthus perulatus* and *E. subsessilis*, both diploid species, is about the same. The stomata of the two differ somewhat in shape. The stomata are much larger in *E. campanulatus*, the octoploid, and its varieties than in the diploids, showing a definite correlation between stomatal size and chromosome number.

Although there is some pairing in threes and fours in *Enkianthus campanulatus*, most of the chromosomes are bivalents in the polyploids.

LITERATURE CITED

BALDWIN, J. T. Cytogeography of *Oxydendrum arboreum*. Bull. Torrey Bot. Club **69**: 134. 1942.

CALLAN, H. G. The cytology of *Gaulthettya wisleyensis* (Marchant) Rehder. Ann. Bot. **5**: 579. 1941.

DARLINGTON, D. C., and E. K. JANAKI-AMMAL. Chromosome atlas of cultivated plants. 379 pp. G. Allen, London. 1945.

FANG, W. P. A preliminary study of the Chinese species of *Enkianthus* Loureiro. Contr. Biol. Lab. Sci. Soc. China **10**: 1935.

HAGERUP, O. Morphological and cytological studies of Bicornes. Dansk. Bot. Ark. **6**: 1. 1928.

———. Zytoökologische Bicornes Studien. Planta **32**: 6. 1941.

HOOKER, J. D. *Enkianthus himalaicus*. Bot. Mag. III. **35**: *pl. 6460*. 1879.

JANAKI-AMMAL, E. K. Polyploidy in the genus *Rhododendron*. Rhododendron Year Book, London **5**: 92–98. 1950.

LI, H. L. Chromosome studies in the azaleas of eastern North America. Am. Jour. Bot. **440**: 8–14. 1957.

LONGLEY, A. E. Chromosomes in *Vaccinium*. Science **66**: 566. 1927.

LOUREIRO, J. *Enkianthus quinqueflorus*. Flora Cochinchinensis **1**: 277. 1790.

MAUDE, P. F. Chromosome numbers in some British plants. New Phytol. **39**: 17. 1940.

NAKAMURA, M. Cytology of *Rhododendron*. Trop. Agric. Tohoku Imp. Univ. **3**: 103. 1931.

NEWCOMER, E. H. Chromosome numbers of some species and varieties of *Vaccinium* and related genera. Proc. Am. Soc. Hort. Sci. **38**: 4–68. 1951.

REHDER, A. *Enkianthus*. *In* Manual of Cultivated Trees and Shrubs. Ed. 2. 729–730. 1940.

———. *Enkianthus*. *In* Bibliography of cultivated trees and shrubs hardy in the cooler temperate regions of the Northern Hemisphere. Arnold Arboretum, Harvard University. Jamaica Plain, Mass. 1949.

SAX, K. Chromosome stability in the genus *Rhododendron*. Am. Jour. Bot. **17**: 247. 1930.

———. The cytogenetics of facultative apomixis in *Malus* species. Jour. Arnold Arb. **40**: 289–297. 1959. [Bark-peel technique, p. 290.]

WANSCHER, J. H. Secondary (chromosome) associations in Umbelliferae and Bicornes. New Phytol. **33**: 58. 1933.

WILSON, E. H. The genus *Enkianthus*. Garden Chron. III. **41**: 311, 344, 363. 1907.

———. *Enkianthus*, page 248 *in* China, Mother of gardens. 4 + 408 pp., map, 6 illustrations. Stratford Co., Boston, Mass. 1929.

———. *Enkianthus*. *In* If I were to make a garden. pp. 205–210. 1931.

WULFF, H. D. Chromosomenstudien an der schleswigholsteinischen Angiospermen Flora, IV. Ber. Deutsch. Bot. Ges. **57**: 424. 1939.